P-nucleotides are so called because they comprise palindromic sequences added to the ends of the gene segments. They are thought to occur in the following way. When the two heptamers of the recombination signal sequence are brought together in the course of DNA rearrangement, the DNA is cleaved precisely between the heptamer and the coding sequence of the gene segments to be joined (see Fig. 3.18, top panel). The two heptamers are then joined to remove the intervening DNA (see Fig. 3.17), but the cleaved ends of the coding segments are not directly ligated to one another. Instead, the cleaved ends are sealed to form hairpins (see Fig. 3.18; second panel) and a single-stranded cleavage subsequently occurs at a random point within the coding sequence so that a single-stranded tail is formed from a few nucleotides of the coding sequence plus the complementary nucleotides from the other DNA strand (see Fig. 3.18; third panel). In most light-chain gene rearrangements, DNA repair enzymes then add the complementary nucleotides to the single-stranded tails and the two double-stranded ends are then rejoined, leaving short palindromic sequences at the joint. In heavy-chain gene rearrangements and in some human light-chain genes, however, N-nucleotides are first added by a quite different mechanism.

N-nucleotides are so called because they are non-template-encoded. They are added to single-stranded ends of the coding DNA after hairpin cleavage by an enzyme called **terminal deoxynucleotidyl transferase (TdT)**. After the addition of up to 20 nucleotides by this enzyme, the two single strands of N-nucleotides form base pairs over a short region. Repair enzymes then trim off any non-matching bases, synthesize complementary bases to fill in the remaining single-stranded DNA, and ligate them to the P-nucleotides (see Fig. 3.18; last three panels). N-nucleotides are absent from mouse light-chain genes because TdT is expressed only for a short period, during the assembly of the heavy-chain genes, which undergo rearrangement before the light-chain genes are assembled.

Since the total number of nucleotides added by these processes is random, the added nucleotides often disrupt the reading frame of the coding sequences beyond the joint. Such frame shifts will normally lead to a non-functional protein—DNA rearrangements leading to such disruptions are known as **non-productive rearrangements**. As roughly two in every three rearrangements will be non-productive, many B cells never succeed in producing functional immunoglobulin molecules, and junctional diversity is therefore achieved only at the expense of considerable wastage. We shall discuss this further when we describe the development of B cells in Chapter 5. The rearrangement of immunoglobulin genes is tightly regulated in such a way as to ensure that each B cell expresses only one rearranged heavy-chain gene and and one rearranged light-chain gene (see Sections 5-7 and 5-8).

3-17 Specialized enzymes are required for somatic recombination of V gene segments.

The complex of several enzymes which act in concert to effect somatic V-region gene recombination is termed the '**V(D)J recombinase**'. This complex comprises mostly the cleavage and repair enzymes present in all cells and required for the normal maintenance of nuclear DNA in any cell type. The first cleavage step, however, requires an additional specialized heterodimeric endonuclease formed from the products of two genes called ***RAG-1*** and ***RAG-2***, for **recombination-activating genes**. *RAG-1* has sequence similarities to a yeast gene, *HRP-1*, and to bacterial **topoisomerases**, which catalyze the breakage and rejoining of DNA. *RAG-1* and *RAG-2* are normally expressed together only in developing lymphocytes. If they are artificially expressed in cells in

culture that do not make antibodies, they can now rearrange introduced unrearranged immunoglobulin gene constructs. Mice with either of the *RAG* gene is knocked out suffer a complete block in primary lymphocyte development at the gene-rearrangement stage (see Section 5-4). A second specialized component of the V(D)J recombinase complex is TdT, discussed in the previous section.

The other components of the recombinase complex are enzymes that normally help repair double-stranded breaks in DNA. They include at least three separate nuclear proteins, one of which is an autoantigen called Ku. Another is the enzyme **DNA-dependent protein kinase**, whose normal role is demonstrated by mutant mice in which it is defective. Such ***scid (severe combined immunodeficient)*** mice cannot join DNA at the junctions between the gene segments encoding the variable region, and so can make only trivial amounts of immunoglobulin or T- cell receptors.

3-18 Rearranged V genes are further diversified by somatic hypermutation.

The mechanisms for generating diversity described so far all take place during the rearrangement of gene segments in the initial development of B cells in primary lymphoid organs. There is an additional mechanism that generates diversity throughout the variable region and which operates on B cells in secondary lymphoid organs after functional antibody genes have been assembled. This process, known as **somatic hypermutation**, introduces point mutations into the variable regions of the rearranged heavy- and light-chain genes at a very high rate, giving rise to mutant immunoglobulin molecules on the surface of the B cells (Fig. 3.19). Some of the mutant immunoglobulin molecules bind antigen better than the original surface immunoglobulin, and B cells expressing them are selected, to mature into antibody-secreting cells, giving rise to a phenomenon called **affinity maturation**, which we will discuss in more detail in Chapters 8 and 9.

Somatic hypermutation occurs when B cells respond to antigen. The immunoglobulin constant-region genes, and other genes expressed in the B cells, are not affected, whereas all rearranged variable region genes are mutated even if they are the result of non-productive rearrangements and are not expressed. The pattern of nucleotide base changes in non-productive variable-region genes illustrates the result of somatic hypermutation without selection for enhanced binding to antigen. The base changes are distributed widely through the V region, but not completely randomly: there are certain 'hotspots' of mutation that indicate a preference for characteristic short motifs of four to five nucleotides, and perhaps also certain ill-defined secondary structural features. The pattern of base changes in the expressed variable-region genes is different. The net result of selection for enhanced binding to antigen is that base changes that alter amino acid sequences are clustered in the CDR1 and CDR2 regions, while silent mutations which preserve amino acid sequence and do not alter structure are scattered throughout the framework regions.

Most of the diversity of antibodies in an adult individual derives from somatic alterations acquired during the lifetime of the individual. The combination of heritable and acquired components of diversity that we have described operates in several mammalian immune systems. Other species achieve a mix of inherited and acquired diversity by different means: birds do not employ somatic recombination or hypermutation to create diversity, but create their repertoires by gene conversion from germline pseudogenes. Overall, it would appear that there is

One class of SCID individuals lack expression of all MHC class II gene products on their cells. This condition is referred to as the **bare lymphocyte syndrome**. Since the thymus also lacks MHC class II molecules CD4 T cells cannot be positively selected and therefore few develop. The antigen-presenting cells in these individuals also lack MHC class II molecules and so the few CD4 T cells that develop cannot be stimulated by antigen. In these individuals, MHC class I expression is normal and CD8 T cells develop normally. However, they suffer severe combined immunodeficiency, illustrating the central importance of CD4 T cells in adaptive immunity to most pathogens. The syndrome is caused by mutations in one of several different genes that regulate MHC class II gene expression rather than in the MHC genes themselves. At least five complementing gene defects have been defined in patients who fail to express MHC class II molecules, which implies that at least five different genes are required for normal MHC class II gene expression. One of these, named the **class II transactivator**, or **CIITA**, is known to be responsible for some cases, while a protein that binds to the MHC class II promoters, called RFX, is defective in others. An understanding of the other genes that cause this defect is still being sought.

A family showing almost complete absence of cell-surface MHC class I molecules has been described. These patients have normal levels of mRNA encoding MHC class I molecules and normal production levels of MHC class I proteins. The defect was shown to be similar to that of the TAP mutant cells we learned about in Section 4-7 and indeed, affected members of this family had mutations in a TAP gene. These people are immunodeficient owing to a lack of CD8 T cells.

An interesting mutant mouse strain called *scid* (because it has a severe combined B- and T-cell immune deficiency) has a defect in the enzyme DNA-dependent kinase, which binds to the end of the double-stranded breaks that occur during the process of antigen receptor gene rearrangement. Many DNA hairpin structures, which are formed when DNA rearrangement is initiated, have been found in T-cell receptor δ-chain genes of immature thymocytes of *scid* mice, and cells from *scid* mice can be rescued by transfection with the catalytic subunit of DNA-dependent protein kinase. Thus, it seems likely that DNA-dependent kinase is involved in resolving the hairpin structure (see Fig. 3.21). Only rare VJ or VDJ joints are seen in *scid* B and T cells, and most of these have abnormal features. These mice therefore produce very few mature B and T cells. Recently, similar abnormal DJ joints have been observed in pre-B cells of some patients with autosomal severe combined immunodeficiency and cells of such patients, like those of *scid* mice, are abnormally sensitive to ionizing radiation. Other patients who appear to have mutations of *RAG-1* or *RAG-2* genes, also show a SCID phenotype.

In patients with **DiGeorge syndrome** the thymic epithelium fails to develop normally. Without the proper inductive environment T cells cannot mature, and both T-cell dependent antibody production and cell-mediated immunity are absent. Such patients have some serum immunoglobulin and variable numbers of B and T cells. The severe combined immunodeficiency diseases abundantly illustrate the central role of T cells in virtually all adaptive immune responses. In many cases B-cell development is normal, yet the response to nearly all pathogens is profoundly suppressed.

10-12 Defective T-cell signaling, cytokine production, or cytokine action can cause immunodeficiency.

As we learned in Chapter 7, virtually all adaptive immune responses require the activation of antigen-specific T lymphocytes and their differentiation